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Acinetobacter Spp.: Distinct Morphology on Eosin Methylene Blue Agar as an Aid to Identification in Drinking Water

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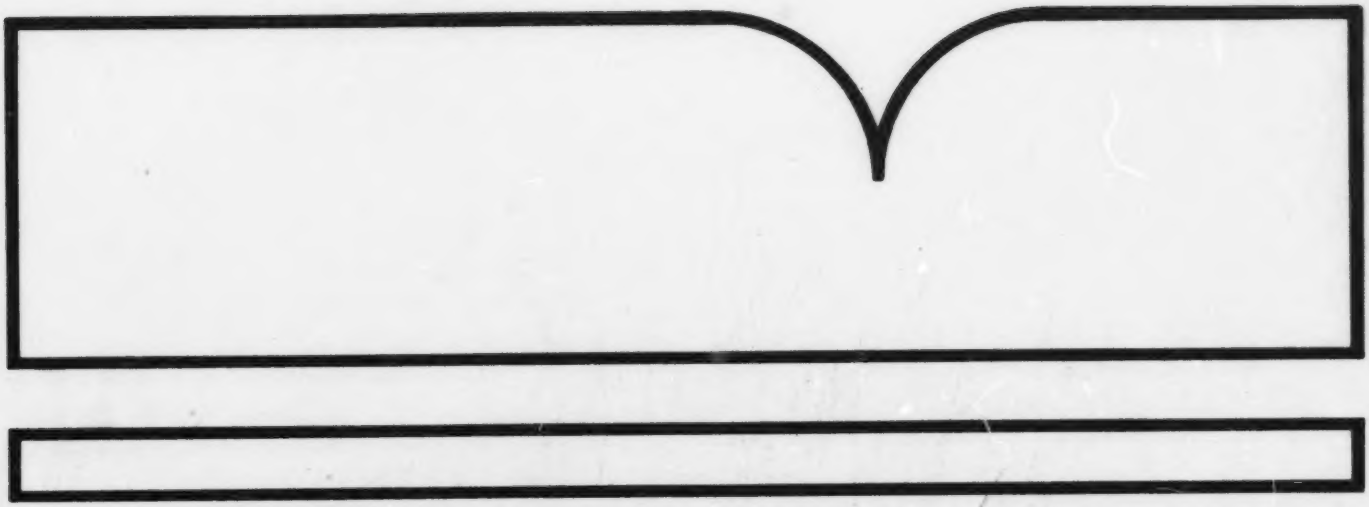

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16. ABSTRACT

Acinetobacter calcoaceticus, frequently found in drinking waters and implicated in nosocomial infections, was presumptively identified by its tiny, blue colonial appearance on Levine eosin methylene blue agar. All of the 33 isolates from drinking water showing this distinctive colonial appearance were identified as A. calcoaceticus.

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Acinetobacter spp.: Distinct Morphology on Eosin Methylene Blue Agar as an Aid to Identification in Drinking Water

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Acinetobacter calcoaceticus, frequently found in drinking waters and implicated in nosocomial infections, was presumptively identified by its tiny, blue colonial appearance on Levine eosin methylene blue agar. All of the 33 isolates from drinking water showing this distinctive colonial appearance were identified as *A. calcoaceticus*.

Acinetobacter calcoaceticus has been reported to be a nosocomial pathogen in 1,372 cases by the Centers for Disease Control (4). This ubiquitous environmental organism has also been reported to cause severe infections, particularly pneumonia and septicemia (6), in humans.

The U.S. Environmental Protection Agency's Drinking Water Research Division investigated the survival of bacteria found in distribution system water during storage, using the standard most-probable-number test for the total coliform group (1). During April 1979, 33 isolates, appearing as tiny, blue colonies on Levine eosin methylene blue agar, which is used in the completed most-probable-number procedure, were picked for subsequent identification. The average turbidity of the samples from which the isolates were made was 1.06 nephelometric turbidity units; there was no residual free chlorine. Characterization of the isolates with the API-20E (Analytab Products, Plainview, N.Y.) procedure gave profile numbers 0005042-03 and 0204042-03; both of these profile numbers identify *A. calcoaceticus* by the October 1977 API chart. When doubtful reactions in a particular API microtube occurred, a standard biochemical test was run for the test in question. The API-20E profile numbers of nine digits are derived from 27 biochemical tests. Salient reactions for *A. calcoaceticus* after 48 h in API microtubes are as follows: hydrolysis of *o*-nitrophenyl-3-galactoside by β-galactosidase (−, [negative]), 100%; ornithine, lysine decarboxylase, and arginine dihydrolase (−), 100%; citrate (+ [positive]), 54%; H_2S (−), 100%; urease (−), 100%; tryptophane deaminase (−), 100%; indole (−), 100%; Voges-Proskauer (+), 17.2%; gelatin (+), 12.6% glucose (+), 87.4%, mannitol (−), 100%; inositol (−), 100%; sorbitol (−), 100%; rhamnose (−), 100%; sucrose (+), 1.2%; melibiose (+), 89.1%; amygdalin (−), 100%; arabinose (+), 79.7%; oxidase (−), 100%; nitrate reduction (+), 3.2%; nitrogen gas (−), 100%; nonmotile, 100%; growth on MacConkey agar (+), 90.5%; glucose oxidation/fermentation O (+), 98.4%; glucose oxidation/fermentation F (−), 100%. The organisms are small, gram-negative rods in the logarithmic growth phase, changing to gram-negative diplococci (resembling *Neisseria*) in the stationary phase (5).

A profile of 0005042-03 gives atypical citrate (−) and Voges-Proskauer (+) reactions, but results are typical for the remaining 24 reactions, which according to API's computerized biochemical statistics is deemed an excellent identification: 1 of 27 strains of *A. calcoaceticus* shows this profile. The other profile, 0204042-03, has a biochemical frequency of 1 of 5 strains for *A. calcoaceticus*, which also is excellent identification with the API's computerized data basis. Some of the tiny, blue colonies were nonreactive in the API system of identification. Nevertheless, they were suspected of being *A. calcoaceticus* because of their microscopic morphology (gram-negative diplococci) and negative oxidase reactions. For these nonreactive strains, the Centers for Disease Control procedure for deoxyribonucleic acid transformation (2), based on Juni's work (3), was used for positive identification. Deoxyribonucleic acid extracts from all suspected *A. calcoaceticus* isolates were coupled to the competent auxotrophs. With the use of the API and deoxyribonucleic acid transformation techniques, all of the 33 blue colony isolates were subsequently identified as *A. calcoaceticus*.

Sanitary microbiology deals primarily with enteric organisms associated with fecal pollution. As these studies are extended to the isolation and identification of nonenteric organisms in drinking water, other microorganisms of po-

tential public health significance are being found. *A. calcoaceticus* is an example of a microorganism that is deemed to be at least an opportunistic pathogen; some clinicians consider it a primary pathogen. Based on our results, it appears that the *Standard Methods* most-probable-number completed test (1), in addition to its routine role for determining the bacteriological quality of drinking water, might be used as an enrichment method to reveal the presence of *A. calcoaceticus* in routine environmental samples.

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